Relationship Between Circulating Vascular Cell Adhesion Molecule-1 and Microvascular Complications in Type 2 Diabetes Mellitus

M. Koga[1], M. Otsuki[2], M. Kubo[1], J. Hashimoto[1], S. Kasayama*[2]

[1]Department of Internal Medicine, Kinki Central Hospital, Itami-city, Hyogo, Japan
[2]Third Department of Internal Medicine, Osaka University Medical School, Suita-city, Osaka, Japan

The soluble form of the vascular cell adhesion molecule-1 (VCAM-1) is detectable in human sera and is elevated in diabetic patients, with unknown clinical significance. In the present study, the relationship between serum soluble VCAM-1 and diabetic microvascular complications (retinopathy, nephropathy, and neuropathy) was evaluated in 95 Japanese patients with Type 2 diabetes mellitus (DM). Serum soluble VCAM-1 concentration was higher in patients with more advanced stages of retinopathy as well as nephropathy. There was a significant correlation between soluble VCAM-1 and $\log_{10}$ (urinary albumin excretion) in 69 patients with normal serum creatinine levels ($r = 0.51$, $p < 0.0001$) and a significant correlation between soluble VCAM-1 and $\log_{10}$ (serum creatinine) in all the patients ($r = 0.83$, $p < 0.0001$). Soluble VCAM-1 concentration was also elevated in patients with neuropathy. There was a significant correlation between soluble VCAM-1 concentration and the number of microvascular complications ($r = 0.59$, $p < 0.0001$). However, multivariate regression analysis revealed that only diabetic nephropathy, was associated with the soluble VCAM-1 concentration. The elevation of circulating VCAM-1 level in diabetic nephropathy may result from underlying systemic endothelial dysfunction, increased VCAM-1 production in damaged renal tubular or glomerular epithelial cells and/or decreased renal clearance of this molecule, depending on the stage of nephropathy. © 1998 John Wiley & Sons, Ltd.

Diabet. Med. **15: 661–667 (1998)**

KEY WORDS **vascular cell adhesion molecule-1; diabetes mellitus; nephropathy; retinopathy; neuropathy**

Received 23 September 1997; revised 6 March 1998; accepted 13 March 1998

Introduction

Mononuclear leucocyte adhesion to vascular endothelium is mediated via adhesion molecules.[1,2] Vascular cell adhesion molecule-1 (VCAM-1) is a member of the immunoglobulin gene superfamily, which was first identified as an adhesion molecule induced on endothelial cells by inflammatory cytokines or lipopolysaccharides.[3] VCAM-1 has been shown to mediate leucocyte binding to the endothelial cells through its interaction with very late antigen-4 (VLA-4), which is selectively expressed on monocytes and lymphocytes but not neutrophils.[4] VCAM-1 has been implicated in inflammatory responses,[5,6] metastasis,[7] and atherosclerosis.[8,9]

The soluble form of VCAM-1 has been detected in endothelial cell culture supernatants and human sera.[10,11] Soluble VCAM-1 is released from cytokine-activated vascular endothelial cells[10] and determination of soluble VCAM-1 may predict dysfunction of vascular endothelium. Soluble VCAM-1 levels are increased in sera from patients with inflammatory diseases, autoimmune disorders, malignancies, and renal failure,[11–15] although its sources and metabolic fate are not known. It has also been reported to be elevated in sera from diabetic patients.[16,17] Recently, Fasching *et al.*[18] have shown that circulating VCAM-1 concentration was higher in Type 1 diabetes mellitus (DM) patients with retinopathy, as well as in those with microalbuminiuria or overt albuminuria, than those without microvascular complications. Schmidt *et al.*[19] have shown that soluble VCAM-1 concentration was elevated in diabetic patients with microalbuminuria. On the other hand, Cominacini *et al.*[20] found that soluble VCAM-1 level in Type 1 and Type 2 DM patients was not significantly different from that in control subjects. Steiner *et al.*[16] also failed to demonstrate any correlation between urinary albumin concentration with soluble VCAM-1 concentration in diabetic patients. These discrepancies may be due to the relatively small number

*Correspondence to: Dr S. Kasayama Third Department of Internal Medicine, Osaka University Medical School, 2-2 Yamada-oka, Suita-city, Osaka 565, Japan. E-mail: kasayama@imed3.med.osaka-u.ac.jp

CCC 0742–3071/98/080661–07$17.50

of the patients or the lack of classification of complications in the previous studies. Certainly it is not known which of the diabetic vascular complications is associated with elevated circulating VCAM-1.

To evaluate the clinical significance of soluble VCAM-1 in DM, more detailed analysis is needed. In the present study, we have examined soluble VCAM-1 concentration in Japanese Type 2 DM patients and analysed its relationship with diabetic vascular complications.

Patients and Methods

Patients

Ninety-five Japanese patients (56 male and 39 female) with Type 2 DM as defined by World Health Organization criteria[21] were studied. The study patients were randomly selected from the patients attending the Kinki Central Hospital or the Osaka University Hospital during the period of August 1995 to December 1996. Patients were 62 ± 9 years old. Mean body mass index was 23.7 ± 3.3 kg m^{-2}, and known diabetes duration was 14 ± 8 years. Mean HbA_{1c} level was 7.5 ± 1.6 %. Fourteen patients (15 %) were treated with diet therapy alone, 43 patients (45 %) with sulphonylureas and 38 patients (40 %) with insulin. Patients with hypertension were using angiotensin-converting enzyme inhibitors, calcium-channel blockers, or alpha- or beta-adrenergic antagonists; those with hypercholesteroaemia pravastatin or simvastatin. Informed consent was obtained from all patients. Patients with active infection, malignant disease or autoimmune disorders were excluded.

All patients had a complete physical and laboratory examination before the study. Diabetic retinopathy was diagnosed by ophthalmologists on fundus examination and photography. There were 37 patients without diabetic retinopathy, 29 patients with non-proliferative diabetic retinopathy, and 29 patients with proliferative diabetic retinopathy.[22] Diabetic nephropathy was classified in the following way. Group I: 37 patients with normoalbuminuria; group II: 24 patients with microalbuminuria; group III: 8 patients with overt albuminuria but not elevated serum creatinine (<110 μmol l^{-1}); group IV: 11 patients with elevated serum creatinine (≥110 μmol l^{-1}) but not receiving haemodialysis or continuous ambulatory peritoneal dialysis; group V: 15 patients receiving haemodialysis. Normoalbuminuria, microalbuminuria, and overt albuminuria were defined on the basis of the determination of urinary albumin excretion (UAE) from at least two subsequent specimens of randomly collected urine samples (expressed as milligrams per mmol creatinine). The definition was according to that of Krolewski *et al.*[23] Normoalbuminuria was defined for male patients as UAE of less than 192 and for female patients as UAE of less than 283. Microalbuminuria was defined as 192 to 3379 for male patients and 283 to 3379 for female patients. Overt albuminuria was defined as UAE of 3380 or higher for both sexes. Diabetic neuropathy was diagnosed in the presence of clinical evidence of peripheral sensorimotor neuropathy plus either abnormal nerve conduction in at least two peripheral nerves or abnormal autonomic nerve function tests.[24] Of the 95 patients, 50 had diabetic neuropathy. There were 21 patients without any microvascular complications, 23 patients with one, 15 patients with two, and the other 36 patients with all three microvascular complications. In addition, there were 27 patients with atherosclerotic vascular diseases: 15 coronary artery disease; 14 cerebral artery disease; 11 peripheral leg artery disease. Among these 27 patients, 10 had multiple atherosclerotic vascular diseases. Clinical characteristics of the patients are shown in Table 1.

Blood samples were obtained by venous sampling. We found no significant change of serum soluble VCAM-1 concentration during the day. From the haemodialysis patients, blood samples were drawn through the existing vascular access just before haemodialysis. Soluble VCAM-1 concentration in sera from haemodialysis fistulae did not differ from that from arm veins.

Table 1. Clinical characteristics of Type 2 DM patients ($n = 95$)

Age	62 ± 9
Sex (M/F)	56/39
Body mass index (kg m^{-2})	24 ± 3
Known diabetes duration (yr)	14 ± 8
Treatment at study (% of patients)	
diet	14 (15)
sulphonylurea	43 (45)
insulin	38 (40)
HbA_{1c}(%)[a]	7.5 ± 1.6
Retinopathy (% of patients)	
nil	37 (39)
non-proliferative	29 (31)
proliferative	29 (31)
Neuropathy (% of patients)	
absent	50 (53)
present	45 (47)
Nephropathy (% of patients)	
group I	37 (39)
group II	24 (25)
group III	8 (8)
group IV	11 (12)
group V	15 (16)
Coronary artery disease (% of patients)	
absent	80 (84)
present	15 (16)
Cerebral artery disease (% of patients)	
absent	81 (85)
present	14 (15)
Peripheral leg artery disease (% of patients)	
absent	84 (88)
present	11 (12)

Data represent means ± SD. Per cent of patients shown in parentheses.
[a]Reference ranges: 4.4–5.8 %.

Laboratory Methods

Serum samples were stored at −20 °C until assay. Serum concentration of soluble VCAM-1 was determined by enzyme-linked immunosorbent assay (ELISA) (R & D Systems, Minneapolis, MN, USA). Dilution curves of serum samples were parallel to those of standards. Intra- and inter-assay coefficients of variation were 3.1 % and 5.9 %, respectively, as determined in representative serum sample. There was no crossreactivity of human IgG, recombinant soluble intercellular adhesion molecule-1 (ICAM-1) or recombinant soluble E-selectin, according to the manufacture's protocol. Control levels of serum soluble VCAM-1 were 597 ± 161 ng ml^{-1} (range 415 to 960 ng ml^{-1}) in our laboratory, which were obtained from 19 healthy volunteers (average age 43 ± 13 yr).

Plasma glucose, HbA_{1c} and serum creatinine levels were determined by standard techniques. Urinary albumin concentration was determined by turbidometric immunnoassay[25] using the specific assay kit (Dia-Iatron, Tokyo, Japan).

Statistical Analyses

All data are shown as means ± SD. To correct for skewed distributions, serum creatinine and urinary albumin concentrations were logarithmically transformed. For statistical analyses, one-way analysis of variance (ANOVA) was used to compare more than two groups. In order to compare two groups, unpaired Student's *t*-test or Welch's correction for unequal variances was used, as appropriate. To analyse the effects of different variables on serum soluble VCAM-1 concentration, univariate regression analysis as well as stepwise multivariate regression analysis were performed with SPSS (Statistical Package for Social Science, SPSS Inc., Chicago, IL, USA) computer program. In the stepwise multiple regression analysis, *p* value for inclusion of the variables was set at 0.05. The statistical differences were considered to be significant at $p<0.05$.

Results

The soluble VCAM-1 concentration in the 58 patients with retinopathy was 1084 ± 501 ng ml^{-1}, significantly higher ($p<0.0001$) than in the 37 patients without retinopathy (647 ± 137 ng ml^{-1}). It was 833 ± 358 ng ml^{-1} in 29 patients with non-proliferative retinopathy and 1336 ± 502 ng ml^{-1} in 29 patients with proliferative retinopathy (Figure 1). Thus, serum soluble VCAM-1 concentration was higher in patients with more advanced stages of retinopathy.

Of the 95 Type 2 DM patients, 26 patients had elevated serum creatinine levels (groups IV and V). Their soluble VCAM-1 concentration was 1501 ± 449 ng ml^{-1}, significantly higher than in 69 patients with normal serum creatinine levels (groups I–III) (693 ± 170 ng ml^{-1}, $p<0.0001$). The soluble VCAM-1 concentration was

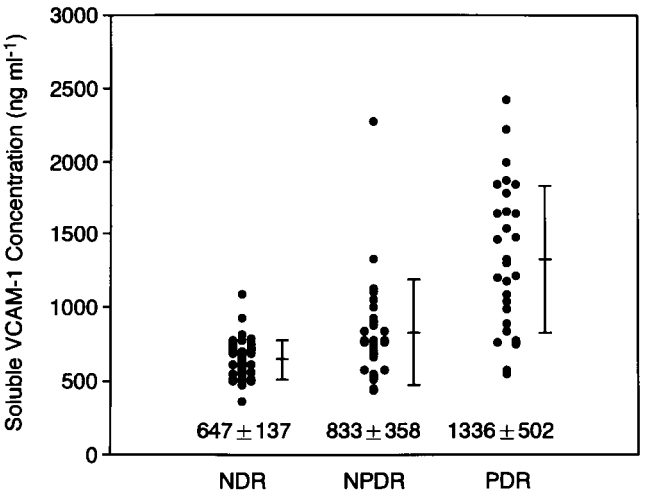

Figure 1. Serum soluble VCAM-1 concentration in Type 2 DM patients in the different groups: patients without diabetic retinopathy (NDR), non-proliferative diabetic retinopathy (NPDR) or proliferative diabetic retinopathy (PDR). Mean (±SD) values are shown to the right of each column of data points. F-ANOVA = 32.4; $p<0.0001$

641 ± 148 ng ml^{-1} in group I, 695 ± 141 ng ml^{-1} in group II, 1015 ± 185 ng ml^{-1} in group III, 1211 ± 325 ng ml^{-1} in group IV, and 1714 ± 411 ng ml^{-1} in group V, which was significantly higher in patients with more advanced stages of nephropathy (Figure 2). In 69 patients who had normal serum creatinine levels (groups I–III), there was a significant correlation between serum soluble VCAM-1 and $\log_{10}$(UAE) ($r = 0.51$, $p<0.0001$; Figure 3). In addition, there was a positive correlation between soluble VCAM-1 and $\log_{10}$ (serum creatinine) in all the 95 Type 2 DM patients ($r = 0.83$, $p<0.0001$; Figure 4).

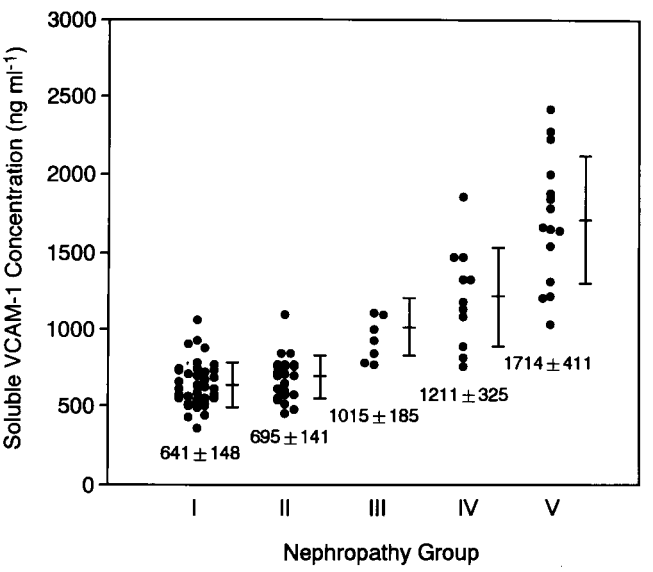

Figure 2. Serum soluble VCAM-1 concentration in Type 2 DM patients. Patients were divided into groups I-V on the basis of the classification of diabetic nephropathy. Mean (±SD) values are shown to the right of each column of data points. F-ANOVA = 68.1; $p<0.0001$

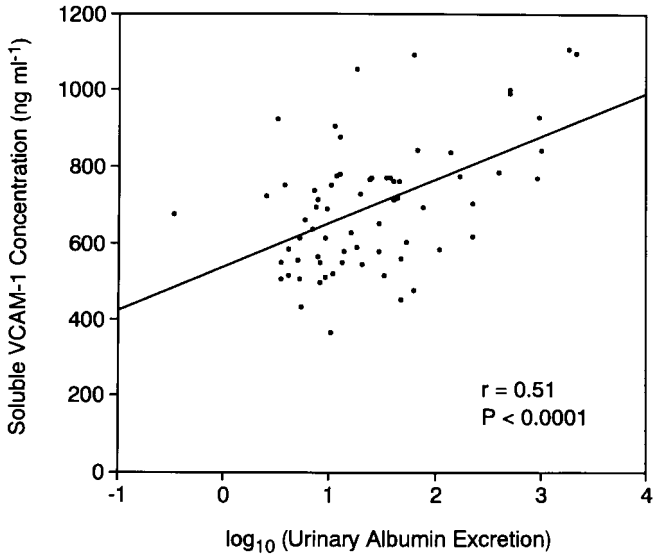

Figure 3. Correlation between $\log_{10}$ (urinary albumin excretion) and serum soluble VCAM-1 concentration in 69 Type 2 DM patients with normal serum creatinine level (groups I–III). Significant correlation was obtained ($r = 0.51$, $p<0.0001$)

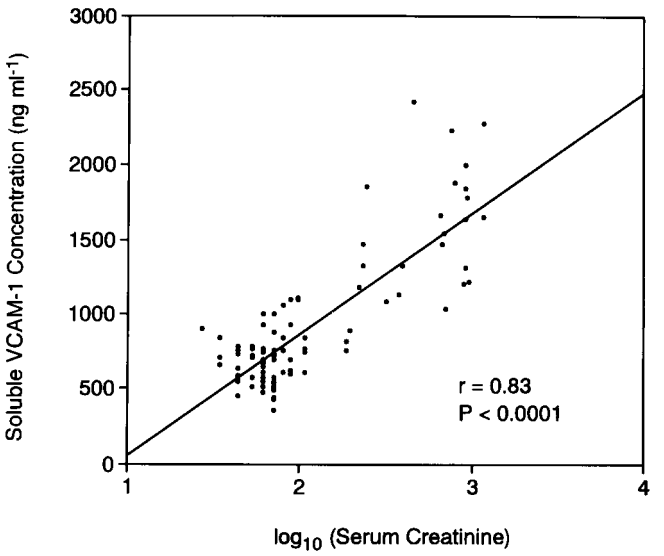

Figure 4. Correlation between $\log_{10}$ (serum creatinine) and serum soluble VCAM-1 concentration in 95 Type 2 DM patients. Significant correlation was obtained ($r = 0.83$, $p<0.0001$)

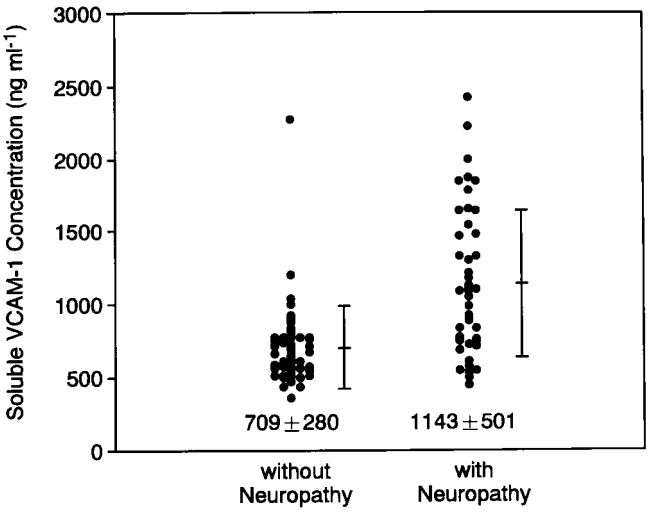

Figure 5. Serum soluble VCAM-1 concentration in Type 2 DM patients without or with diabetic neuropathy. Mean (±SD) values are shown to the right of each column of data points. The difference between each group is $p<0.0001$

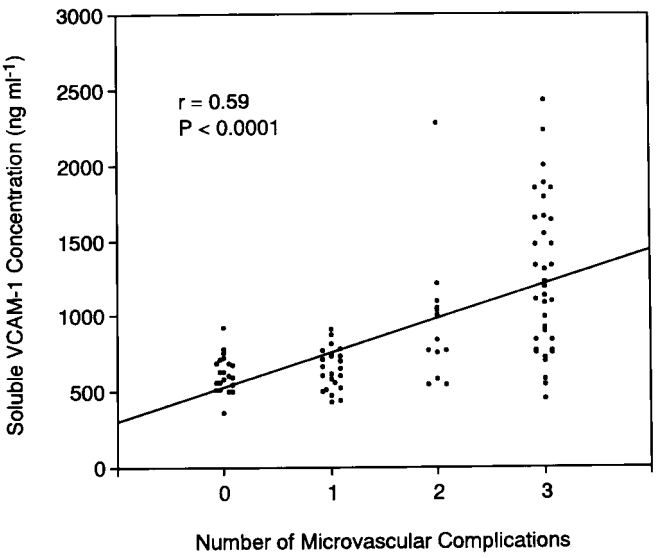

Figure 6. Correlation between serum soluble VCAM-1 concentration and number of microvascular complications in Type 2 DM patients. Significant correlation was observed ($r = 0.59$, $p<0.0001$)

In 45 patients with diabetic neuropathy, soluble VCAM-1 concentration was 1143 ± 501 ng ml^{-1}, significantly higher ($p<0.0001$) than in 50 patients without neuropathy (709 ± 280 ng ml^{-1}; Figure 5).

Soluble VCAM-1 concentration in 21 patients without any microvascular complications was 622 ± 122 ng ml^{-1}. In contrast, the soluble VCAM-1 concentration in 23 patients with one microvascular complication was 651 ± 135 ng ml^{-1}; 936 ± 422 ng ml^{-1} in 15 patients with two complications; and 1244 ± 501 ng ml^{-1} in 36 patients with all three complications (Figure 6). There was a significant correlation between soluble VCAM-1 and the number of microvascular complications ($r = 0.59$, $p<0.0001$).

Univariate regression analysis (Table 2) showed that nephropathy, retinopathy, neuropathy, atherosclerotic vascular diseases, known diabetes duration, and age were associated with soluble VCAM-1 concentration. Smoking habit, body mass index, treatment at study, HbA_{1c}, and sex were not. To evaluate the independent effect of the related variables on soluble VCAM-1 concentration, stepwise multivariate regression analysis was performed. The results showed that only nephropathy was significantly associated with serum soluble VCAM-1 concentration (Table 3). The other variables including retinopathy, neuropathy, atherosclerotic vascular dis-

Table 2. Univariate regression analysis of the effects of variables on serum soluble VCAM-1 concentration

Variable	Correlation coefficient	*p* value
Nephropathy (1 = group I; 2 = group II; 3 = group III; 4 = group IV; 5 = group V)	0.83	<0.0001
Retinopathy (1 = nil; 2 = non-proliferative; 3 = proliferative)	0.62	<0.0001
Neuropathy (0 = absent; 1 = present)	0.48	<0.0001
Atherosclerotic vascular diseases (0 = absent; 1 = present)	0.36	0.0004
Known diabetes duration (yr)	0.33	0.001
Age (yr)	0.24	0.018
Smoking (0 = no; 1 = yes)	0.17	0.09
Body mass index (kg m^{-2})	0.17	0.11
Treatment at study (1 = diet alone; 2 = sulphonylurea; 3 = insulin)	0.17	0.11
HbA_{Ic}(%)	0.059	0.57
Sex (0 = male; 1 = female)	0.047	0.65

Univariate regression analysis was performed on 95 Type 2 DM patients.

Table 3. Stepwise multivariate regression analysis of the effects of variables on serum soluble VCAM-1 concentration

Variable	Partial regression coefficient	*f* value	*p* value
Nephropathy	252.9	208.4	<0.0001

Multivariate regression analysis was performed on 95 Type 2 DM patients. R^2 for the entire model = 0.69. Explanatory variables: nephropathy (1, group I; 2, group II; 3, group III; 4, group IV; 5, group V); retinopathy (1, nil; 2, nonproliferative; 3, proliferative); neuropathy (0, absent; 1, present); atherosclerotic vascular diseases (0, absent; 1, present); known diabetes duration (years); age (years).

eases, known diabetes duration, and age did not enter the regression model.

Discussion

VCAM-1 has been shown to be expressed in various cells including endothelial cells, vascular smooth muscle cells, resident macrophages, lymphoid dendritic cells, and renal glomerular and tubular epithelial cells.[1,26,27] Although soluble VCAM-1 is found in the circulation of normal individuals and patients with various disorders,[11–20] its clinical significance remains unclear.

We have recently reported that circulating VCAM-1 may be a marker of atherosclerosis in Type 2 DM patients with normal serum creatinine levels.[28] In the present study, examining Type 2 DM patients including those with increased serum creatinine levels, we demonstrated that soluble VCAM-1 concentration was elevated in patients with any diabetic microvascular complication. This is similar to a recent observation demonstrating that soluble VCAM-1 concentration was higher in Type 1 DM patients with retinopathy or nephropathy.[18] Our study clearly showed that soluble VCAM-1 concentration was significantly higher in patients with more advanced stages of retinopathy or nephropathy and was also elevated in patients with neuropathy, with a positive correlation between soluble VCAM-1 and the number of diabetic microvascular complications. In univariate regression analysis, atherosclerotic vascular diseases, known diabetes duration, and age were also associated with the soluble VCAM-1 concentration. Thus, soluble VCAM-1 concentration was found to be elevated in Type 2 DM patients with older age, longer disease duration, and micro- and macrovascular complications. However, multivariate regression analysis demonstrated that diabetic nephropathy was only associated with serum soluble VCAM-1 concentration in the Type 2 DM patients. Therefore, in the Type 2 DM patients serum soluble VCAM-1 concentration was found to be dependent on the state of diabetic nephropathy.

Soluble VCAM-1 concentration in patients with increased serum creatinine levels was much higher than those with normal serum creatinine levels and was the highest in the haemodialysis patients (with a positive correlation between serum soluble VCAM-1 and serum creatinine. Patients with chronic renal failure have the increased levels of soluble VCAM-1[11,15] and haemodialysis reduced its levels, suggesting that the kidney is a route of elimination of the soluble VCAM-1. However, we have found urinary VCAM-1 to be elevated in diabetic patients with increased serum creatinine levels (unpublished observation). Thus, it is unlikely that impaired renal clearance of soluble VCAM-1 molecules is the only cause of the elevated soluble VCAM-1 in the patients with increased serum creatinine levels.

In patients with normal serum creatinine levels, soluble VCAM-1 concentration positively correlated with $\log_{10}$(UAE). This suggests soluble VCAM-1 concentration increases with progression of nephropathy, even in patients with normal serum creatinine levels. Soluble VCAM-1 concentration in patients with microalbuminuria or overt albuminuria was significantly higher than that in patients with normoalbuminuria, compatible with reports by Schmidt *et al.*[19] that soluble VCAM-1 concentration was elevated in diabetic patients with microalbuminuria. VCAM-1 has been found to be expressed in proximal tubular cells and glomerular epithelial cells of kidney and VCAM-1 positive tubular cells were more common in diabetic nephropathy than in amyloidosis and gouty nephropathy.[29] Thus, the elevated soluble VCAM-1 concentration in patients with microalbuminuria or overt albuminuria might be caused partly by increased production and secretion of VCAM-1 to the circulation from kidney. In addition, it has been shown that early diabetic nephropathy is associated with systemic endothelial dysfunction,[30] suggesting that generalized

vasculopathy in patients with early diabetic nephropathy contributes to the increase of soluble VCAM-1 concentration. In this context, advanced glycation endproducts[17] but not high glucose[31] have been shown to enhance VCAM-1 expression in cultured endothelial cells. Recently, it has been shown that soluble VCAM-1 stimulates angiogenesis.[32] It leads us to speculate that the elevated soluble VCAM-1 in patients with diabetic nephropathy may cause and/or worsen diabetic retinopathy.

In conclusion, circulating soluble VCAM-1 concentration was elevated in Type 2 DM patients with older age, longer disease duration, microvascular complications or atherosclerotic vascular diseases. In multiple regression analysis, however, we demonstrated for the first time that only diabetic nephropathy is associated with the soluble VCAM-1 concentration. It may reflect systemic endothelial dysfunction, increased VCAM-1 expression in renal tubular and glomerular epithelial cells, and/or decreased renal clearance of soluble VCAM-1, which are dependent on the stage of nephropathy.

Acknowledgements

This work was supported by Grants from the Ministry of Education, Science and Culture of Japan (to S.K.) and from Enami Memorial Foundation of Diabetes Research (to M.K.). We would like to thank our clinical staff for assisting the clinical management of the study patients, M. Mukai for performing the statistical analysis of the data and K. Tsujii for secretarial assistance in preparing the manuscript.

References

1. Bevilacqua MP, Nelson RM, Mannori G, Cecconi O. Endothelial-leukocyte adhesion molecules in human disease. *Ann Rev Med* 1994; **45**: 361–378.
2. Springer TA. Adhesion receptors for the immune system *Nature* 1990; **346**: 425–434.
3. Osborn L, Hession C, Tizard R, Vassallo C, Luhowskyj S, Chi-Rosso G, Lobb R. Direct expression cloning of vascular cell adhesion molecule 1, a cytokine-induced endothelial protein that binds to lymphocytes. *Cell* 1989; **59**: 1203–1211.
4. Elices MJ, Osborn L, Takada, Y, Crouse C, Luhowskyj S, Hemler ME, Lobb R. VCAM-1 on activated endothelium interacts with the leukocyte integrin VLA-4 at a site distinct from the VLA-4/fibronectin binding site. *Cell* 1990; **60**: 577–584.
5. Briscoe D, Shoen FJ, Rice GE, Bevilacqua MP, Ganz P, Pober JS. Induced expression of endothelial-leukocyte adhesion molecules in human cardiac allografts. *Transplantation* 1991; **51**: 537–539.
6. Koch AE, Burrows JC, Haines GK, Carlos TM, Harlan JM, Leibovich J. Immunolocalization of endothelial and leukocyte adhesion molecules in human rheumatoid and osteoarthritic synovial tissues. *Lab Invest* 1991; **64**: 313–320.
7. Rice GE, Bevilacqua MP. An inducible endothelial cell surface glycoprotein mediates melanoma adhesion. *Science* 1989; **246**: 1303–1306.
8. Cybulsky MI, Gimbrone MA Jr. Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. *Science* 1991; **251**: 788–791.
9. O'Brien KD, Allen MD, McDonald TO, Chait A, Harlan JM, Fishbein D, *et al*. Vascular cell adhesion molecule-1 is expressed in human coronary atherosclerotic plaques. Implications for the mode of progression of advanced coronary atherosclerosis. *J Clin Invest* 1993; **92**: 945–951.
10. Pigott R, Dillon LP, Hemingway IH, Gearing AJH. Soluble forms of E-selectin, ICAM-1 and VCAM-1 are present in the supernatants of cytokine activated cultured endothelial cells. *Biochem Biophys Res Commun* 1992; **187**: 584–589.
11. Gearing AJH, Hemingway I, Pigott R, Hughes J, Rees AJ, Cashman SJ. Soluble forms of vascular adhesion molecules, E-selectin, ICAM-1, and VCAM-1: pathological significance. *Ann NY Acad Sci* 1992; **667**: 324–331.
12. Wenisch C, Myskiw D, Parschalk B, Hartmann T, Dam K, Graninger W. Soluble endothelium-associated adhesion molecules in patients with Graves' disease. *Clin Exp Immunol* 1994; **98**: 240–244.
13. Wellicome SM, Kapahi P, Mason JC, Lebranchu Y, Yarword H, Haskard DO. Detection of a circulating form of vascular cell adhesion molecule-1: raised levels in rheumatoid arthritis and systemic lupus erythematosus. *Clin Exp Immunol* 1993; **92**: 412–418.
14. Banks RE, Gearing AJH, Hemingway IK, Norfolk DR, Perren TJ, Selby PJ. Circulating intercellular adhesion molecule-1 (ICAM-1), E-selectin and vascular cell adhesion molecule-1 (VCAM-1) in human malignancies. *Br J Cancer* 1993; **68**: 122–124.
15. Rabb H, Calderon E, Bittle PA, Ramirez G. Alterations in soluble intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 in hemodialysis patients. *Am J Kidney Dis* 1996; **27**: 239–243.
16. Steiner M, Reinhard KM, Krammer B, Ernst B, Blann AD. Increased levels of soluble adhesion molecules in types 2 (non-insulin dependent) diabetes mellitus are independent of glycaemic control. *Thromb Haemost* 1994; **72**: 979–984.
17. Schmidt AM, Hori O, Chen JX, Li JF, Crandall J, Zhang J, *et al*. Advanced glycation endproducts interacting with their endothelial receptor induce expression of VCAM-1 in human endothelial cells and in mice: a potential mechanism for the accelerated vasculopathy of diabetes. *J Clin Invest* 1995; **96**: 1395–1403.
18. Fasching P, Veitl M, Rohac M, Streli C, Schneider B, Waldhäusl W, Wagner OF. Elevated concentrations of circulating adhesion molecules and their association with microvascular complications in insulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 1996; **81**: 4313–4317.
19. Schmidt AM, Crandall J, Hori O, Cao R, Lakatta E. Elevated plasma levels of vascular cell adhesion molecule-1 (VCAM-1) in diabetic patients with microalbuminuria. A marker of vascular dysfunction and progressive vascular disease. *Br J Haematol* 1996; **92**: 747–750.
20. Cominacini L, Pasini AF, Garbin U, Davoli A, De Santis A, Campagnola M, *et al*. Elevated levels of soluble E-selectin in patients with IDDM and NIDDM. *Diabetologia* 1995; **38**: 1122–1124.
21. World Health Organization Study Group. *Diabetes Mellitus*. WHO Technical Report Series 727. Geneva: WHO, 1985: 1–113.
22. Aiello LM, Cavallerano JD. Ocular complications of diabetes mellitus. In: Kahn CR, Weir GC, eds. *Joslin's Diabetes Mellitus*, 13th edn. Malven: Lea & Febiger, 1994: 771–793.

23. Krolewski AS, Lori MB, Krolewski M, Quinn M, Warram JH. Glycosylated hemoglobin and the risk of microalbuminuria in patients with insulin-dependent diabetes mellitus. *N Engl J Med* 1995; **332**: 1251–1255.
24. Kroc Collaborative Study Group. Blood glucose control and the evolution of diabetic retinopathy and albuminuria: a preliminary multicenter trial. *N Engl J Med* 1984; **311**: 365–372.
25. Watts GF, Bennett JE, Rowe DJ, Morris RW, Gatling W, Shaw KM, Polak A. Assessment of immunochemical methods for determining low concentrations of albumin in urine. *Clin Chem* 1986; **32**: 1544–1548.
26. Rice GE, Munro JM, Corless C, Bevilacqua MP. Vascular and nonvascular expression of INCAM-110. *Am J Pathol* 1991; **138**: 385–393.
27. Bevilacqua MP. Endothelial-leukocyte adhesion molecules. *Ann Rev Immunol* 1993; **11**: 767–804.
28. Otsuki M, Hashimoto K, Morimoto Y, Kishimoto T, Kasayama S. Circulating vascular cell adhesion molecule-1 (VCAM-1) in atherosclerotic NIDDM patients. *Diabetes* 1997; **46**: 2096–2101.
29. Seron D, Cameron, JS, Haskard DO. Expression of VCAM-1 in the normal and diseased kidney. *Nephrol Dial Transplant* 1991; **6**: 917–922.
30. Jensen T, Bjerre-Knudson J, Feldt-Rasmussen B, Deckert T. Features of endothelial dysfunction in early diabetic nephropathy. *Lancet* 1989; **i**: 461–463.
31. Baumgartner-Parzer SM, Wagner L, Gessl A, Waldhäusl W. Modulation by high glucose of adhesion molecule expression in cultured endothelial cells. *Diabetologia* 1995; **38**: 1367–1370.
32. Koch AE, Halloran MM, Haskell CJ, Shah MR, Polverini PJ. Angiogenesis mediated by soluble forms of E-selectin and vascular cell adhesion molecule-1. *Nature* 1995; **376**: 517–519.

